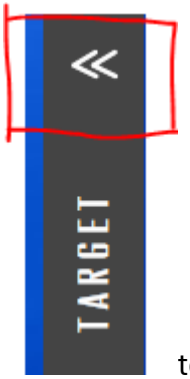
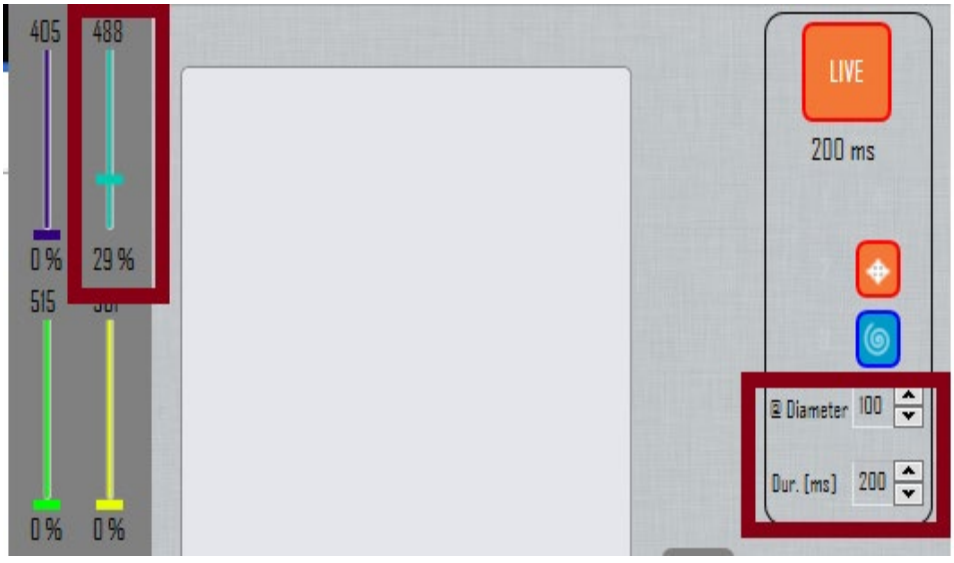
Live-SR spinning disk confocal _ Confocal imaging with FRAP

1. Start instrument power switches 1, 2, 3 and workstation 4.
2. Make sure the laser LightPath on the back of microscope body is set to 90C (iLAS).
3. Start MetaMorph (MM).
4. Select FRAP-CSU DAPI/Fp/RFP/Cy5 based on sample labels. Start MM Live to find the sample.
5. Select "Target" 

to open the FRAP "Live" window. Click on 

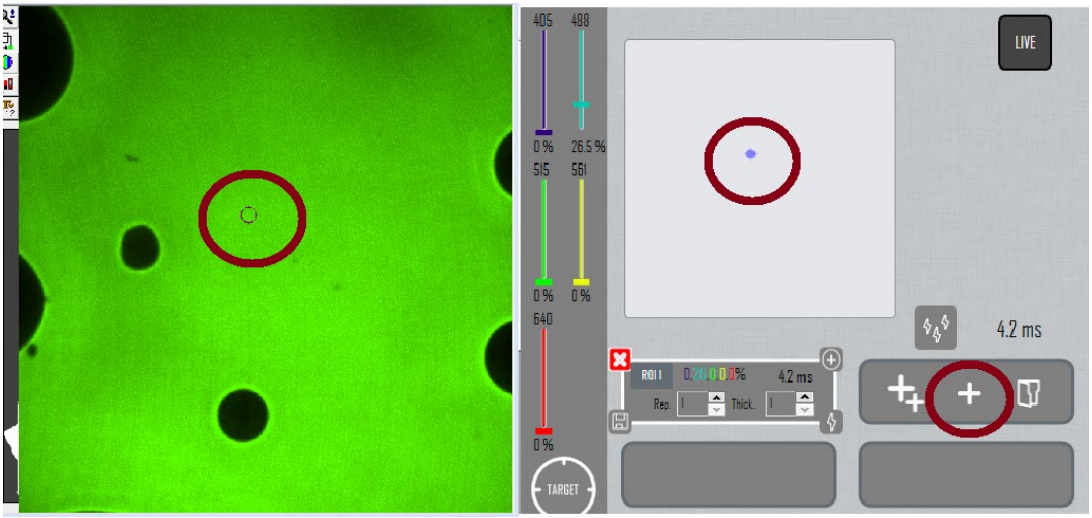
to start live view of FRAP. On this window, set the diameter of the FRAP point and Dur (ms). set the laser power for FRAP.



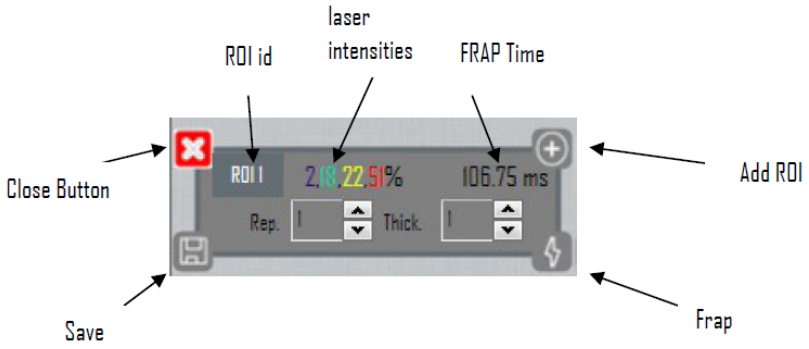
6. Move the mouse to MM image live window and click to have a live view of Photobleaching effect on the sample.
7. If you need calibration for precision of the FRAP location, refer to the end of the last session of this document.

8. To add region for FRAP, select drawing of ROI in MM.

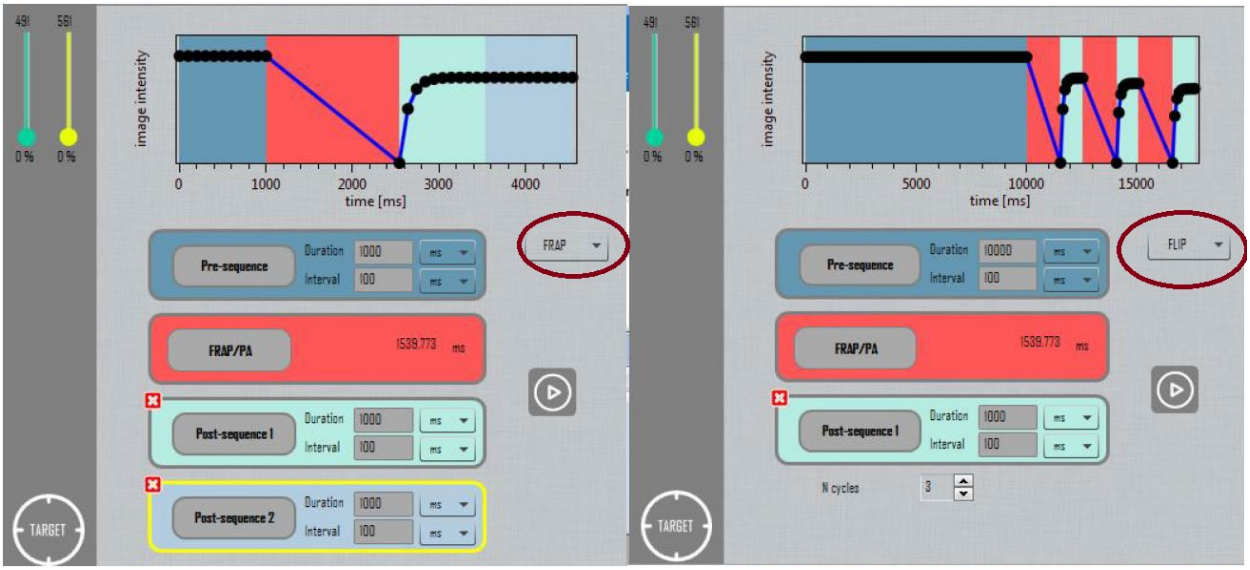
9. Keep MM Live on, and add ROI to image window. When the edge of the ROI blinks at white color, click on  to add active ROI. The ROI will be shown in the FRAP window. Or if multiple ROI have been displayed in the MM image widow, click on  to add all ROIS onto FRAP window.



10. Save ROI. “Close button” will erase this ROI.



11. To setup the FRAP during image acquisition, click on  to open FRAP MDA window which directly programs MDA in MM. For onetime photobleaching, select FRAP. For repetitive photobleaching and observation, select FLIP.



12. For FRAP:
    a. Pre-sequence:
        i. Interval: E.g. for the MDA acquistion, it takes 1s to complete one time point acquisition. You may enter 100ms (<1s) for maximum speed acquisition or enter 2S to get one second waiting time between each acquisition.
        ii. Duration: interval x number of frames to be used before photobleaching. E.g. if one would like to capture two frames' image before photobleaching, enter "200ms" for "Duration" if "100ms" is set for the "interval".
    b. FRAP/PA: shows FRAP duration for all the ROIs.
    c. Post -Sequence: same as the Pre-Sequence. One can add multiple post-sequences with different duration and interval.
13. For FLIP, enter N cycles to observe repetitive photobleaching and recovery.
14. In MDA, set up the image acquisition as per normal.
15. In FRAP window, hit  to loads this information on MM MDA which is shown on "journal".
16. In MDA, start "acquire".

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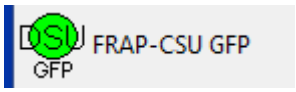
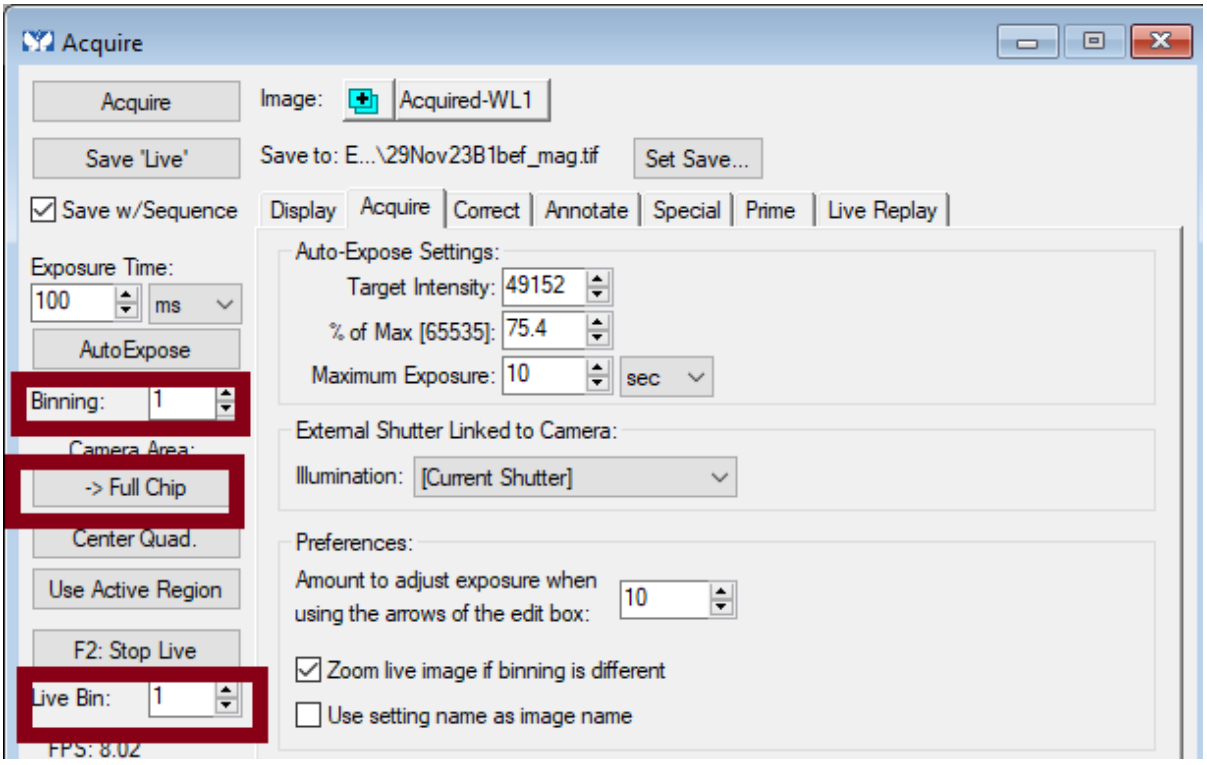
FRAP calibration.

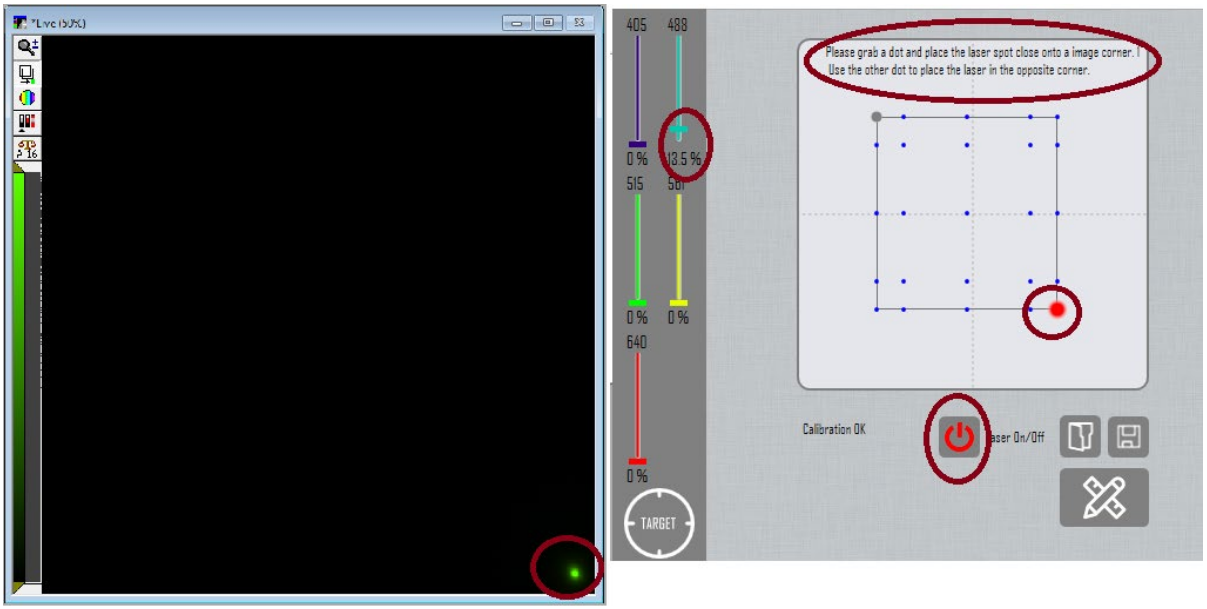
1. Choose window 
2. Mount a green Chroma slide onto stage.
3. “FRAP-CSU GFP” 

+ live  sample. Stop live.
4. Check there is no binning and full chip is used.

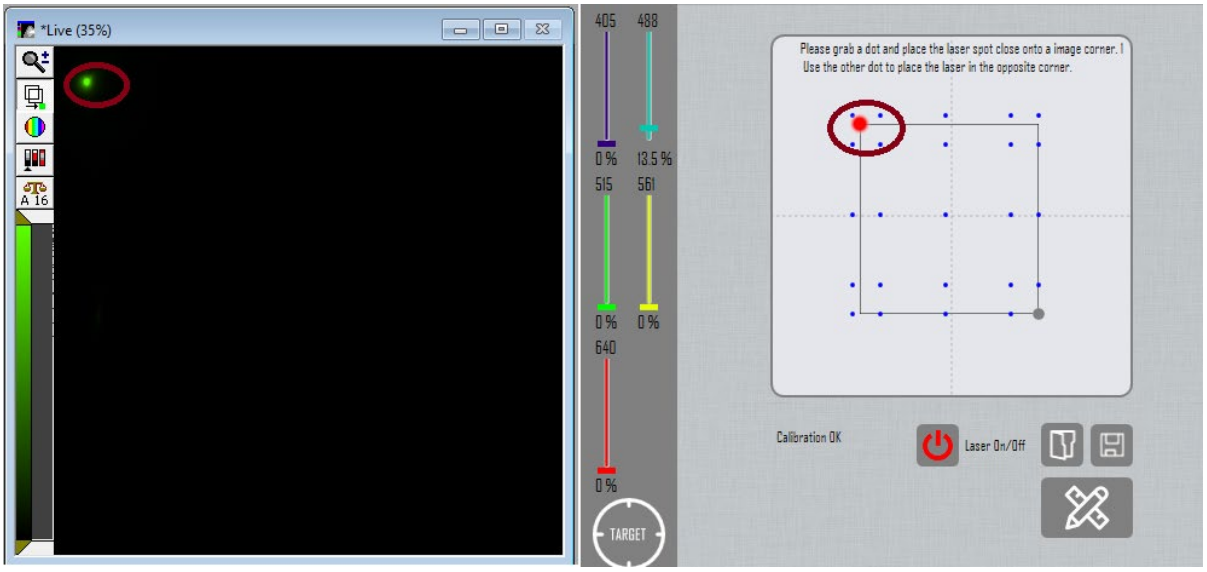


5. On FRAP setting window, turn on laser and adjust laser intensity. Start  . Grab a dot and place the laser spot close onto an image corner. You will be able to see a bright dot on the image window. Change the focus until you get to the smallest spot

size.



6. use the other dot to place the laser in teh opposite corner.



7. Click on  to start calibration.
8.  save the calibration for further use (to reload/open).